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## REMARKS/ARGUMENTS

1. Remarks on the Amendment

Applicants appreciate the Examiner's allowance of Claims 1-5, 7-14, 16-19, 21-28, 30-53, 55-65 and 67-71.

Claims 15, 29, 54 and 66 have been amended to more specifically define Applicants' claimed invention. Applicants respectfully submit that no new matter has been added by the amendments.

There are now total of 69 claims pending.

2. Response to the rejection of Claims 15, 29, 54 and 66 based upon 35 USC §112

Claims 15, 29, 54 and 66 stand rejected under 35 USC 112, second paragraph. This rejection is respectfully traversed by the amendments.

Applicants have amended Claims 15, 29, 54 and 66 as the Examiner suggested.

For clarification of the file record, Applicants respectfully point out that the derivatives of poly (DTE carbonate) are understood by those skilled in the art. Among four articles submitted with the response submitted on August 28, 2003, two articles published before the filing date of the instant application showed poly (DTE carbonate) derivatives; one showed the use of poly (DTH carbonate), and the other showed the use of poly (DTB carbonate), poly (DTH carbonate), and poly (DTO carbonate). Furthermore, the third publication was September 2000, however, it was a 3 year study, as stated in the title of the article, using a group of polymers carrying an alkyl ester pendent chain. These polymers were available 3 years prior to the publication date of September 2000.

Moreover, one of the submitted abstracts is from an article (the full article is submitted herein, *Biomaterials*, 1999 Dec. 20 (23-24):2203-12) which has cited

eight other publications (reference numbers 4, 5 and 11-16) related to this series of tyrosine-derived polycarbonates in their synthesis, physicomechanical properties, sterilization, surface chemistry, degradation and biocompatibility. These cited eight references were published from 1992 to 1997, all prior to the filing date of the instant application.

Applicants further point out that Claims 15, 29, 54 and 66 are dependent claims of allowed independent Claims 1, 18, 36, and 42, respectively. The claimed poly (DTE carbonates) and their derivatives are clearly under the scope of the independent claims.

As stated previously, Applicants have amended these rejected claims as suggested by the Examiner, and Applicants will address the above-discussed issue in a subsequent patent application.

Applicants respectfully request withdrawal of the rejection of Claims based upon 35 U.S.C. §112, second paragraph.

It is respectfully submitted that Claims 1-5, 7-19, 21-35, 36-41 and 42-71 the pending claims, are now in condition for allowance and such action is respectfully requested. Applicant's Agent respectfully requests direct telephone communication from the Examiner with a view toward any further action deemed necessary to place the application in final condition for allowance.

3/4/2004
Date of Signature

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# Small changes in polymer chemistry have a large effect on the bone–implant interface: evaluation of a series of degradable tyrosine-derived polycarbonates in bone defects

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**Abstract**

In a series of homologous, tyrosine-based polycarbonates, small changes in the chemical structure of the polymer pendent chain were found to affect the bone response in a long-term (1280 d) implantation study. Identically sized pins, prepared from poly(DTE carbonate), poly(DTB carbonate), poly(DTH carbonate), and poly(DTO carbonate) were implanted transcortically in the proximal tibia and the distal femur of skeletally mature New Zealand White Rabbits. The tissue response at the bone–implant interface was characterized in terms of the absence of a fibrous capsule (direct bone apposition, indicative of a bone bonding response) or the presence of a fibrous capsule (referred to as the encapsulation response). The relative frequency of direct bone apposition versus encapsulation was recorded for each polymer throughout the entire period of the study. While all four polymers were tissue compatible, there was a correlation between the chemical structure of the pendent chain and the type of bone response observed, with poly(DTE carbonate) having the highest tendency to elicit direct bone apposition. Based on in vivo degradation data and the ability of model polymers with carboxylate groups at their surface to chelate calcium ions, it is proposed that the ability of poly(DTE carbonate) to bond to bone is caused by the facile hydrolysis of the pendent ethyl ester groups which creates calcium ion chelation sites on the polymer surface. The incorporation of calcium chelation sites into the chemical structure of an implant material appears to be a key requirement if direct bone apposition/bone bonding is desired. This study demonstrates that very subtle changes in the chemical composition of an implant material can have significant effects on the long-term tissue response in a clinically relevant model.


*Keywords:* Tyrosine-based polycarbonate; Biomaterial; Prosthesis–bone interface; Bone–material interactions; Degradable polymer; Poly(DTE carbonate); Structure–property relationships; Calcium ion chelation; Bone apposition; Bone bonding

## 1. Introduction

The tissue response to polymeric biomaterials of widely varying chemical structures is often remarkably similar. Whether the device is made from a polysiloxane, polyurethane, Dacron™, Teflon™, or polyethylene, the response elicited in vivo is usually characterized by encapsulation by a persistent fibrous capsule of a dense array of fibroblasts, collagen, and inflammatory cells. This rather ubiquitous response is generally referred to as a 'foreign body response'. Ratner, in a widely cited review paper, has referred to such an unspecific, material-chemistry independent response as the 'blah' response [1].

For many years, some believed that the only possible response at the bone–implant interface was the formation of a fibrous tissue layer that effectively separated the bone from the implant [2]. Later, it was recognized that certain inorganic materials such as hydroxyapatite and other calcium phosphates, silica based Ca–P containing glasses, and even titanium can exhibit direct bone apposition to the implant. Strictly speaking, the term 'bone apposition' describes the absence of an intervening fibrous tissue layer between the implant and the surrounding bone, as observed at the light microscopic level

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in histological sections [2]. In contrast, the terms 'bioactive' and 'bone-bonding' are sometimes used interchangeably to indicate that the tissue response resulted in some degree of either physical/mechanical interlocking between bone and the implant or that chemical bonding occurred between bone and reactive groups at the implant surface [3–6].

Most of the synthetic degradable polymers that are currently being investigated as alternatives to metallic and ceramic orthopedic implants and, increasingly, as tissue engineering scaffolds for bone regeneration, do not exhibit bone apposition but are encapsulated by fibrous tissue. In particular, bone formed around the widely studied polylactides, polyglycolides, and polydioxanone is separated from the implant by a thin layer of connective tissue [3–5]. A notable exception is the copolymer of poly(ethylene oxide) and poly(butylene terephthalate) known under the tradename Polyactive® [7]. For this polymer, direct bone apposition was observed histologically at the bone–implant interface. The mechanism proposed to be responsible for this response centers on the finding that Polyactive® induces the formation of surface hydroxycarbonate apatite, possibly because of the polymer's affinity for calcium ions [8]. More recently, a tyrosine-based polycarbonate, poly(DTE carbonate), was shown in the canine bone chamber model to also exhibit a remarkable degree of direct bone apposition [4].

In an effort to further study the effect of a polymer's chemical structure on the bone–implant interface, we synthesized a family of homologous, tyrosine-derived polycarbonates, including poly(DTE carbonate). The test polymers had identical chemical backbone structures and varied only in the structure of the alkyl ester pendent chain which was increased in length from ethyl (2 carbons), to butyl (4 carbons), hexyl (6 carbons) and octyl (8 carbons) as shown in Fig. 1. This series of polymers has a defined gradient of material properties and seemed to be particularly suitable to establish relationships between chemical structure, polymer properties, and the corresponding biological response [9,10]. For example, as reported by Ertel et al. [11], lengthening the pendent chain decreases the glass transition temperature, increases the surface hydrophobicity of the polymer, and affects fibroblast attachment, proliferation and spreading in vitro.

This series of tyrosine-derived polycarbonates has already been the subject of several investigations concerning their synthesis, physicomechanical properties, sterilization, surface chemistry, degradation, and biocompatibility [4,5,11–16]. These polymers are amorphous materials that can be processed by solvent casting, extrusion, compression and injection molding, or various fiber spinning techniques. Depending on the processing conditions, pins with ultimate tensile strength and Young's Modulus upward of 50 MPa and 2 GPa, respectively, have been obtained. When implanted, tyrosine-based polycarbonates degrade via hydrolysis on the order of months to years to natural metabolites.

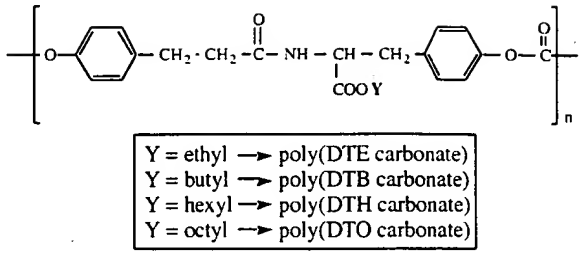


Fig. 1. The chemical structure of tyrosine-derived polycarbonates consists of desaminotyrosyl-tyrosine alkyl esters with different ester groups: ethyl (E), butyl (B), hexyl (H), and octyl (O). The abbreviations for these monomers are represented by DTE, DTB, DTH, and DTO.

Using this well-characterized family of tyrosine-based polycarbonates made it possible to investigate whether small variations in the chemical structure of a polymeric implant can affect the tissue response at the bone–implant interface. For this purpose, extruded pins of poly(DTE carbonate), poly(DTB carbonate), poly(DTH carbonate), and poly(DTO carbonate) were: (i) implanted transcortically in bone defects in the rabbit's distal femora and proximal tibiae permitting histological comparisons of the bone–implant interface as a function of incremental changes in polymer structure and time; and (ii) implanted subcutaneously over the paravertebral muscles in the rabbit and evaluated for changes and differences in pin physical appearance, mechanical properties, and molecular weight loss. In this clinically relevant model system, the transcortical implants were followed over a post implantation period of 1090 d (3 yr) and the subcutaneous implants were followed over a post implantation period of 1280 d (3.5 yr). At the later timepoints, the implants were in advanced stages of degradation. This permitted examination of the differences between the short-term and long-term tissue responses to these degradable polymers.

In addition, a set of new model polymers was prepared with controlled amounts of free carboxylate groups. These model polymers allowed us to test the hypothesis that the formation of carboxylate groups during the degradation of tyrosine-derived polycarbonates can lead to the chelation of calcium ions at the polymer surface, providing a potential mechanism contributing to the apparent bone-bonding behavior observed for poly(DTE carbonate).

## 2. Methods

### 2.1. Implant preparation

Poly(DTE carbonate), poly(DTB carbonate), poly(DTH carbonate), and poly(DTO carbonate) were

synthesized according to previously published procedures [11,15]. Polymer rods 2 mm in diameter were extruded under an argon blanket with a MiniMax extruder system (Custom Scientific Inc., NJ). Poly(DTE carbonate), poly(DTB carbonate), poly(DTH carbonate), and poly(DTO carbonate) were extruded at 190°C, 180°C, 160°C, and 140°C respectively, which are temperatures approximately 90–100°C above the $T_g$ of each material.

Each smooth rod was cut into $2.0 \pm 0.1$ cm lengths using a sharp blade, dimensioned, and weighed (typically 80 mg). Acceptable variations in diameter for bone and subcutaneous implants were, respectively, $2.0 \pm 0.05$ and $2.0 \pm 0.15$ mm. Pins were individually packaged, sterilized with ethylene oxide (Anprolene; Anderson Products, Chapel Hill, NC), and allowed to degas for at least two weeks under vacuum at room temperature. The (weight average) molecular weight ($M_w$) and polydispersity (PD) of the implanted pins were: poly(DTE carbonate) $M_w = 109\,000$, PD = 1.8; poly(DTB carbonate) $M_w = 120\,000$, PD = 1.8; poly(DTH carbonate) $M_w = 147\,000$, PD = 1.7; and poly(DTO carbonate) $M_w = 137\,000$, PD = 1.7.

### 2.2. In vivo testing

A group of 36 male skeletally mature New Zealand white rabbits (3.0–3.5 kg) was divided into eight experimental groups that were sacrificed at 90, 180, 270, 360, 540, 720, 1090, and 1280 d. Using previously published procedures [17], 2 mm diameter bicortical defects were created with a drill in the bone of the distal femora and proximal tibiae. In each rabbit, pins of poly(DTE carbonate), poly(DTB carbonate), and poly(DTO carbonate) were randomly assigned to the implant sites. The implants were press-fit into the bone defects. Excess material protruding from the implant site was trimmed to the bone. At 90 and 180 d two poly(DTH carbonate) pins were used instead of poly(DTB carbonate) pins to provide overlap with a previous study of poly(DTH carbonate) in the same model [5]. Pins of all test polymers were also placed and retrieved from a subcutaneous site over the paravertebral muscles for characterizing changes in molecular weight, appearance, and physicomechanical properties. Because pin retrieval disrupted the implant/tissue interface, and because detailed histological observations in soft tissue had been reported previously [18], no histological analysis was performed for the subcutaneous implant sites.

### 2.3. Polymer degradation

Changes in pin appearance, molecular weight loss, mass loss, and physicomechanical properties over the implantation period were determined from the subcutaneously implanted pins. At retrieval, each pin was visually inspected, weighed, dimensioned, and mechanically tested. The test specimens were then dried under vacuum for two weeks, weighed again, and prepared for molecular weight determination.

Polymer molecular weight ($M_w$ and $M_n$) was determined by gel permeation chromatography (GPC) on a system consisting of a Perkin–Elmer pump (Perkin Elmer; Model 410), a Waters differential refractometer (Waters; Model 410), and a Perkin–Elmer Model 600 computerized data station. Two 30 cm PL-gel columns (Polymer Laboratories) with $10^3$ and $10^5$ angstrom pores, respectively, were operated in series at a flow rate of 1 ml/min in THF. Molecular weights were calculated relative to polystyrene standards (Polymer Laboratories Inc.).

The ultimate tensile strength and Young's modulus of the polymer pins were measured in tension using a servohydraulic biaxial mechanical testing system coupled with a computerized data acquisition system (MTS). The pins were gripped with custom-designed pin vises (gauge length 1.0 cm). The pins were tested submerged in a 37°C water bath at a 1% strain rate.

### 2.4. Polymer biocompatibility and the bone–implant interface

The distal and proximal femora and tibiae implants were retrieved from each animal. Care was taken to assure that the implants were not disturbed by leaving sufficient host bone around the implant. The samples were fixed in 10% phosphate-buffered formalin, dehydrated in a graded ethanol series, and polymethylmethacrylate embedded for undecalcified light microscopy analysis [19]. The medial aspects of each PMMA embedded bone were sectioned (0.5 mm thick) with a diamond saw (Buehler). The sections were glued to Plexiglas slides, hand ground, and polished to a thickness of approximately 30 μm. At least three sections were prepared per implant site. Histological sections were stained with Stevenel's blue and Van Gieson's picrofuchsin so that bone stains red, osteoid stains green, and fibrous tissue stains blue. The histological sections were evaluated in light of the overall cellular response as an indicator of material biocompatibility, e.g. levels and types of inflammatory cells and/or osteolysis of surrounding bone. Next, the bone–implant interface around the circumference of each implanted pin was evaluated in light of whether (a) a fibrous tissue layer separated the implant from the surrounding bone or (b) direct bone apposition was the dominant feature of the bone–implant interface.

### 2.5. Synthesis of model polymers for the evaluation of $Ca^{2+}$ chelation

The synthesis of model polymers followed a two step process: First, a copolymer of DTE and DTBn (where the

Y in Fig. 1 is a benzyl pendent chain) of desired composition was prepared by phosgenation of an appropriate molar ratio of these two monomers using published procedures [15]. Then, the benzyl ester pendent chain was removed by selective catalytic hydrogenolysis using a palladium catalyst. This resulted in the formation of a desired proportion of carboxylate groups. For this study, model polymers in which 25 and 50 mol% of all pendent chains were free carboxylate groups were synthesized. These polymers were designated as poly ($DTE_{0.75}$-*co*-$DT_{0.25}$ carbonate) and poly ($DTE_{0.50}$-*co*-$DT_{0.50}$ carbonate) respectively. In a typical hydrogenolysis, copolymer (30 g) was placed in a Parr Pressure Hydrogenator along with 250 ml of ultrapure (amine free) DMF and 2 g of a solid powder of 5% palladium on barium sulfate (catalyst). The Parr Hydrogenator was pressurized to 55 psi of hydrogen and agitated for 24 h at room temperature. The completion of the debenzylation reaction was confirmed by $^1H$ NMR. The reaction mixture was filtered using a celite bed to remove the catalyst and the polymer was further purified by repeated precipitations in chilled isopropanol.

### 2.6. Simulated body fluid (SBF)

Within a 1.0 l volumetric flask, the following materials were dissolved in 900 ml of water: $Na_2SO_4$ (0.071 g), $K_2HPO_4$ (0.176 g), $NaHCO_3$ (0.353 g), $CaCl_2$ (0.368 g), $MgCl{\cdot}6H_2O$ (0.305 g), NaCl (7.99 g), KCl (0.224 g), Tris(hydroxymethyl-aminomethane) (6.06 g), HCl (1.0 N) (45 ml). The solution was then adjusted by the addition of 2 N NaOH to a pH of 7.4 and the volume was made up to 1.0 l with distilled water. The solution was sterilized by membrane filtration (0.22 μm filter). This composition of SBF is similar to other compositions in the literature [8].

### 2.7. Incubation of model polymers in SBF

Model polymers having no free carboxylate groups, 25% free carboxylate groups, and 50% free carboxylate groups were used, i.e., poly(DTE carbonate), poly-($DTE_{0.75}$-*co*-$DT_{0.25}$ carbonate), and poly($DTE_{0.50}$-*co*-$DT_{0.50}$ carbonate). Films of each model polymer were prepared by solvent casting from a 90 : 10 solution (v/v) of methanol/methylene chloride having a polymer concentration of 10% (w/v). After casting, films were dried under vacuum at room temperature for 1 week. Films were cut into 1.5 cm × 1.5 cm strips. Each strip was placed into an individual glass scintillation vial. To each sample, 10 ml of simulated body fluid (SBF) was added, the vials were sealed, and incubated at 37°C. After incubation for 0, 3, 7, and 14 d, the samples were washed with pure water three times and dried under vacuum at room temperature for 4 h.

### 2.8. Surface analysis by electron spectroscopy for chemical analysis (ESCA/XPS)

After incubation for the specified periods, films of each model polymer were analyzed by electron spectroscopy for chemical analysis (ESCA/XPS) with a Kratos XSAM-8000 instrument. ESCA measurements were carried out at room temperature. The ESCA core level spectra were recorded using unmonochromatized Mg $K_\alpha$ radiation (1254 eV). The pass energy was 80 eV for the survey spectra and 20 eV for the high resolution studies. The build-up of positive charge on the polymer surface was neutralized by using an electron floodgun. The peak areas were converted into atomic concentrations using the following sensitivity factors: 0.25 for carbon, 0.66 for oxygen, 0.42 for nitrogen and 1.58 for calcium.

## 3. Results and discussion

### 3.1. Polymer biocompatibility and the bone–implant interface

The pins were placed transcortically through the distal end of the femur or the proximal end of the tibia. After fixing in a block of polymethylmethacrylate, sagittal histological section were cut through the medial portions of the cortex. Cross section of each pin of the test polymers were observed within and adjacent to the long bone's cortex. At timepoints as early as 90 d (3 months) and as late as 1090 d (3 yr), all the polymeric implants were found to be surrounded by bone without any obvious deleterious effects such as bone resorption or large concentrations of inflammatory cells at the implant site. Poly(DTE carbonate), poly(DTB carbonate), poly(DTH carbonate), and poly(DTO carbonate) were all osteocompatible according to traditional definitions [2,20].

A fundamental difference was seen, however, amongst the polymers at the bone–pin interface—some pins were encapsulated with fibrous tissue (an encapsulation response) whereas others exhibited predominantly direct apposition of bone to the implant surface. Figs. 2 and 3 illustrate typical histological sections for each of the materials over the course of the study. As is evident in Figs. 2 and 3 and in Table 1, the two responses seen at the bone–implant interface were quite distinct. The encapsulation response was distinguished by an organized fibrous capsule that ranged between 3 and 30 cell layers. The capsule lined the entire circumference of the implant and effectively separated the implant from the surrounding bone. In contrast, in those specimens where bone apposition was observed, the circumference of the implant was devoid of an organized fibrous capsule. The tissue responses could be readily classified as either an 'encapsulation response' or as 'direct bone apposition'. The intermediate case of partial encapsulation (presence

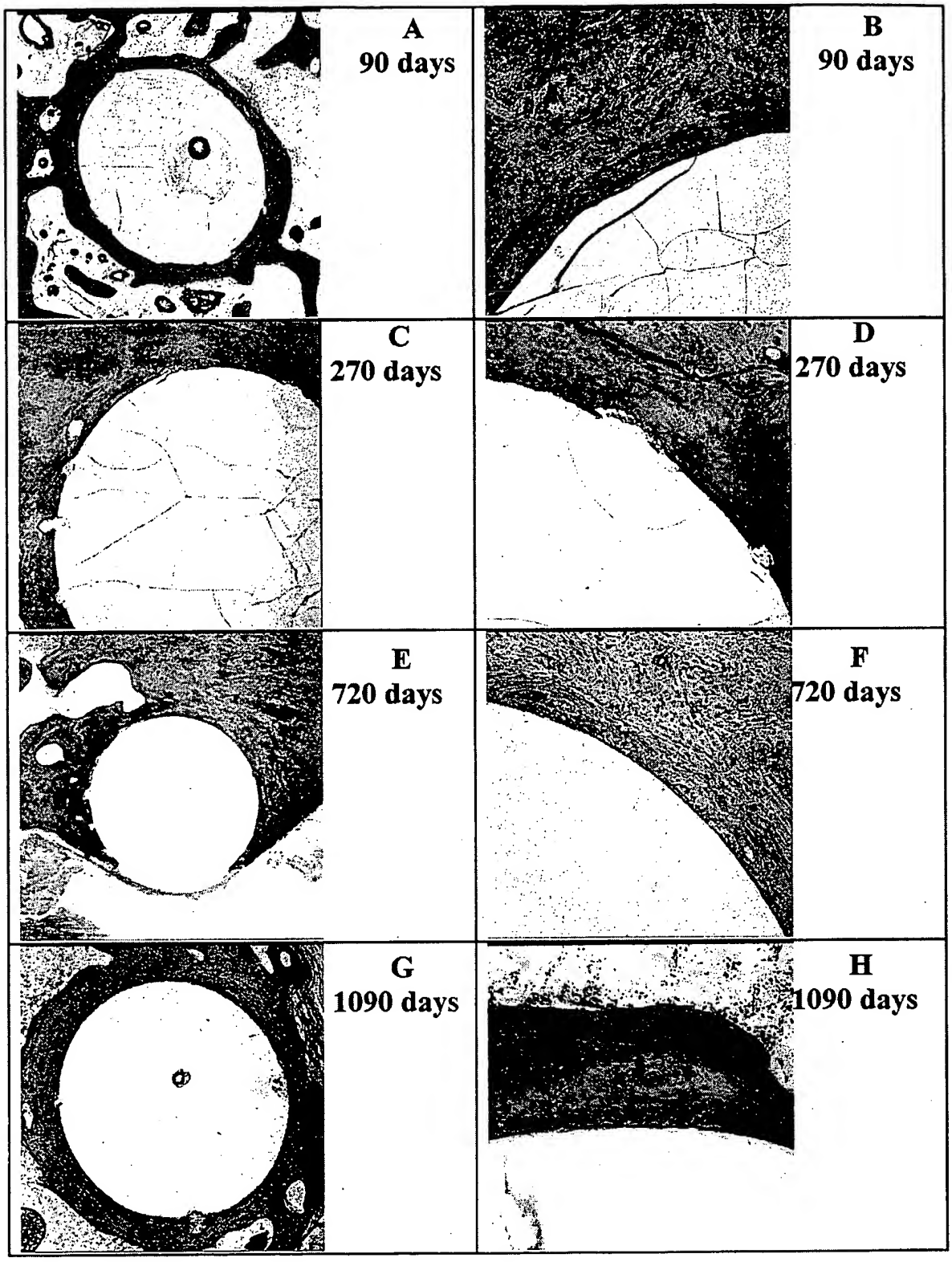


Fig. 2. Interface between pins of poly(DTE carbonate) and bone taken from time points ranging from 90 to 1090 d (3 yr) post implantation. Mineralized bone is stained red, fibrous tissue is blue, and osteoid is stained in a green hue. The histological sections shown illustrate the 'bone apposition' response seen in 73% of all poly(DTE carbonate) implant sites. The bone apposition response was remarkably consistant throughout the entire period of this study. A, C, E and G: low magnification views of the pin circumference 90 d (3 months), 270 d (9 months), 720 d (24 months), and 1090 d (36 months) post implantation, respectively. B, D, F and H: high magnification detail of the bone–implant interface 90 d (3 months) 270 d (9 months), 720 d (24 months), and 1090 d (36 months) post implantation, respectively.

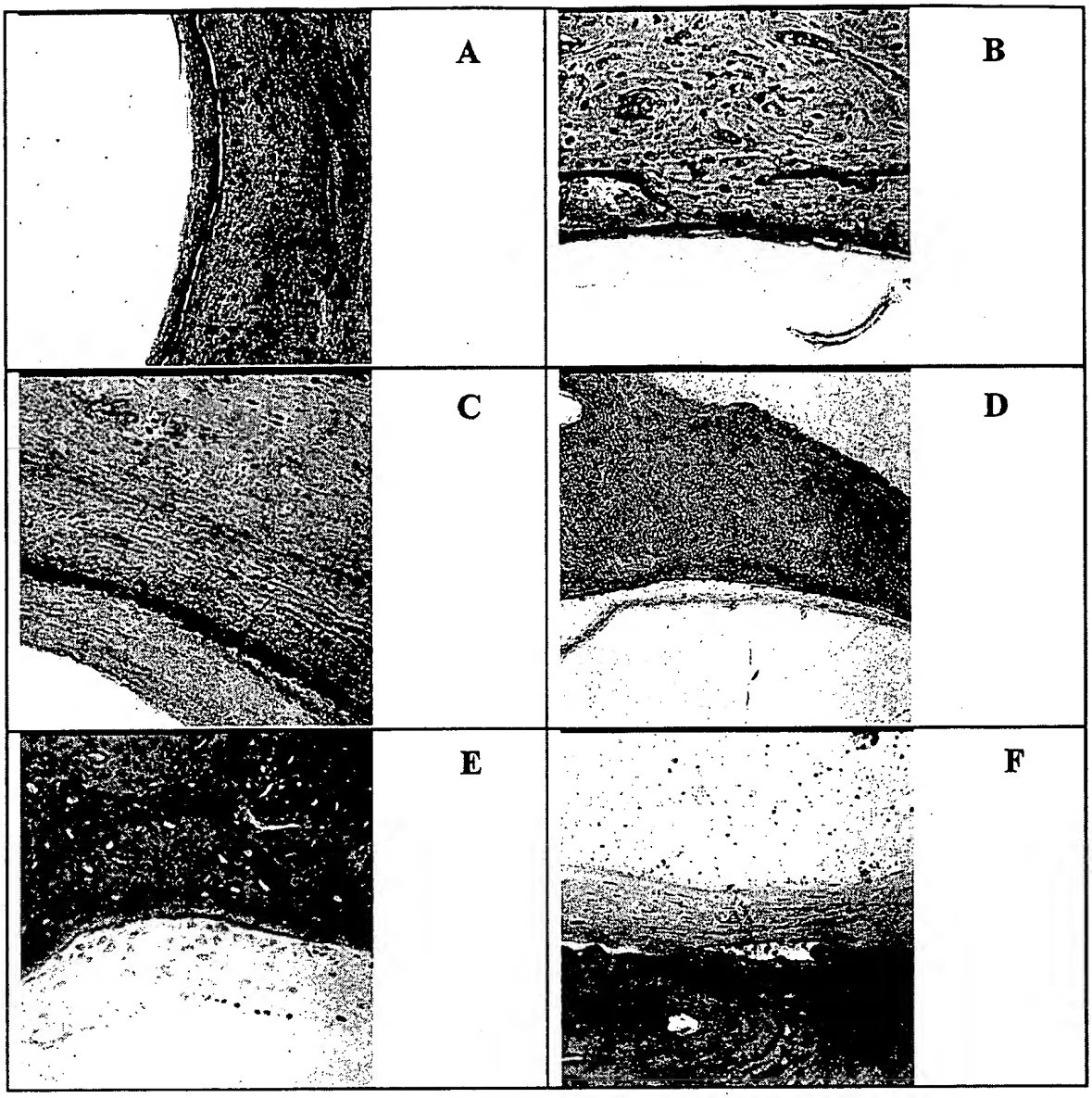


Fig. 3. Representative histological sections illustrating the 'encapsulation response'. This response was observed for all test polymers except poly(DTE carbonate) in the majority of all implant sites. This figure illustrates the variability of the 'encapsulation response'. Mineralized bone is stained red, fibrous tissue is blue, and osteoid is stained in a green hue. (A) Quiescent fibrous capsule of intermediate thickness, shown here for a poly(DTO carbonate) implant at 90 d post implantation. Note the absence of inflammatory cells and the lack of evidence for bone resorption. A large number of implant sites demonstrated this response. (B) Very thin fibrous capsule, observed here for a poly(DTB carbonate) implant after 90 d post implantation. This type of capsule was also observed at some of the poly(DTE carbonate) pins. (C) Very thick fibrous capsule, observed at the interface with a poly(DTO carbonate) implant 270 d post implantation. In spite of the pronounced thickness of the capsule, there is no evidence of an inflammatory response or bone resorption. Such thick capsules were never observed at the interface of poly(DTE carbonate) implants, but were common for poly(DTO carbonate). (D) Quiescent and mature fibrous capsule at the interface with a poly(DTB carbonate) implant 720 d (2 yr) post implantation. (E) Thick, loosely organized and faintly stained fibrous tissue at the interface of a poly(DTO carbonate) pin 1090 d (3 yr) post implantation. (F) Thick, and quiescent fibrous capsule with aligned fibroblasts observed for a poly(DTB carbonate) interface 1090 d (3 yr) post implantation.

of an organized fibrous capsule along only a fraction of the implant circumference) was very rarely observed.

While Figs. 2 and 3 provide representative histological sections, Table 1 lists the frequency by which the encapsulation or bone apposition responses were observed. Most striking was the bone response to poly(DTE carbonate) where direct bone apposition to the implant was the defining feature in 73% of the retrieved implants (22 of 30 pins). These data confirm earlier observations of direct bone apposition with poly(DTE carbonate) in the canine bone chamber model. Those implants that did exhibit an encapsulation response, tended to have thin

Table 1
Frequencies of direct bone apposition and encapsulation responses at the bone–implant interface

| | Poly(DTE carbonate) | | Poly(DTB carbonate) | | Poly(DTO carbonate) | |
|---|---|---|---|---|---|---|
| | Bone apposition (%) | Encapsulation (%) | Bone apposition (%) | Encapsulation (%) | Bone apposition (%) | Encapsulation (%) |
| Short term 0–180 d ($n$ = 10) | 60 | 40 | 30 | 70 | 20 | 80 |
| Long term 270–1090 d ($n$ = 26) | 80 | 20 | 17 | 83 | 16 | 84 |
| Overall results ($n$ = 36) | 73 | 27 | 21 | 79 | 17 | 83 |

Note: *Bone apposition* responses were reported when an organized fibrous tissue layer could not be identified at the light microscopic level at the bone–implant interface.

*Encapsulation* responses were reported when a fibrous capsule encompassed the implanted pin.

capsules of less than 10 cell layers. In contrast, as the length of the pendent chain was increased to butyl and octyl, less bone apposition was observed and the predominant response was the formation of a fibrous capsule. Particularly noteworthy is the dramatic difference in the predominant biological response elicited by poly (DTE carbonate) and poly(DTB carbonate) as these two polymers have very closely matched chemical structure and material properties. Poly(DTB carbonate) exhibited direct bone apposition to the surface in only 21% of the implant sites. The frequency of bone apposition for poly(DTO carbonate) further decreased to 17% of the implants. For all polymers tested, except poly(DTE carbonate) the majority of all implant sites exhibited well established fibrous capsules that tended to be in excess of 10 cell layers. Clearly, in this family of tyrosine-derived polycarbonates, the predominant response elicited at the bone–implant interface was significantly influenced by a relatively minor modification of the polymer structure.

### 3.2. Temporal changes in the tissue response

The long duration of this study made it possible to examine the changes in the tissue response over time. Table 1 provides a listing of the bone–implant response frequencies for both short-term and long-term timepoints. Because of the small number of samples (especially in the listing for the early time points where $n$ = 10) the data in Table 1 must not be overinterpreted. The key point emerging from this study is that the basic features of the bone response were established early on and remained unchanged throughout the remainder of the study. Already within the first 180 days, the increased frequency of direct bone apposition exhibited by poly(DTE carbonate) was clearly discernable.

### 3.3. In vivo polymer degradation

The differences in the bone–implant interface described above could be a result of differences in polymer degradation kinetics, release of degradation products, and/or differences in mechanical properties. To account for these variables, the in vivo degradation characteristics of the polymer pins were followed, using the subcutaneously implanted specimens for which degradation data are available up to 1280 d (3.5 yr) post implantation.

At implantation, the pins made from each material were essentially identical in dimensions and appearance: 2 cm long, 2 mm in diameter, and transparent with a light golden hue. When explanted, all subcutaneous pins were encased in a thin fibrous membrane. By 270 d, some changes in appearance became noticeable: All pins with the exception of poly(DTE carbonate) pins had become opaque and had started to deform/bend. By 1280 d (3.5 yr), poly(DTE carbonate) pins still maintained their shape and only the inside of the pin had become opaque. In contrast, the pins of all other test polymers had changed shape and had became opaque throughout their entire volume to various degrees.

The observed tendency of pins to deform/bend is probably related to the release of residual stress associated with the laboratory-scale fabrication of the pin specimens. The tendency to deform correlated with the differences in the polymers' glass transition temperature ($T_g$). Poly(DTE carbonate) had the highest $T_g$ (81°C) of all polymers and had therefore the lowest tendency to deform. It is reasonable to expect that the deformation of pins made of poly(DTB carbonate), poly(DTH carbonate), and poly(DTO carbonate) will be less pronounced when pins are extruded in commercial-scale equipment under optimized conditions. The changes in opacity are most probably related to the uptake of small amounts of water (less than 4 wt%) into the polymer matrix. Contrary to devices made of poly(lactic acid) or poly(glycolic acid), pins made of tyrosine-derived polycarbonates do not swell noticeably during their degradation process.

While this study provided insufficient data points to establish correlations between temporal changes in physical appearance of the pins and the polymer structure, it is important to note that during the first 180 d of the study, neither deformations nor changes in opacity were

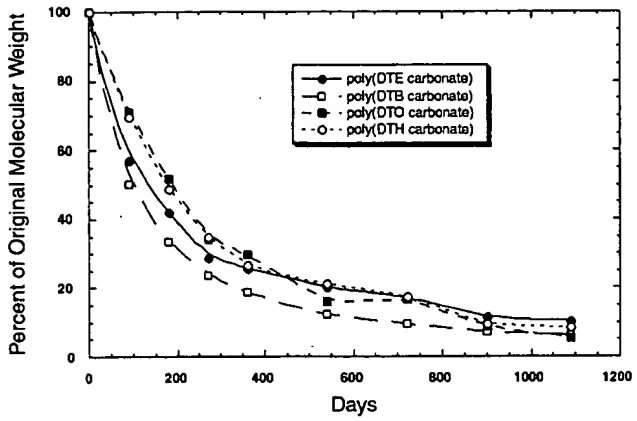


Fig. 4. Molecular weight retention as a function of implantation time of tyrosine-derived polycarbonates. All four of the polymers degraded at comparable rates, indicating that the length of the pendent chain did not affect polymer backbone cleavage. At 1090 d, all the polymers reached a $M_w$ lower than 15% of the original $M_w$. The intitial $M_w$ of poly(DTE carbonate), poly(DTB carbonate), poly(DTH carbonate) and poly(DTO carbonate) was 109, 120, 147 and 137 kDa respectively.

observed. As shown in Table 1, significant differences in the bone response to the test polymers were already clearly established at a time when physical changes in pin shape or opacity can be excluded as contributing factors.

Sustained polymer chain cleavage, i.e., hydrolysis of the carbonate bond, occurred at comparable rates for each of the polymers tested (Fig. 4). Varying the pendent chain did not significantly alter the degradation rate of this family of polymers. This is likely because the polymers have comparable water uptake ranging from 2–4% of the polymer dry weight. Though we measured significant losses in molecular weight, the pins did not change in mass through 1090 d. Mass loss was only observed at the 1280 d time point and was limited to about 2% of the initial weight. In spite of massive backbone degradation to less than 15% of the initial molecular weight, the polymers had yet to degrade to the point where significant amounts of low molecular weight, soluble species would be formed. Thus, the study can be divided into two key periods: the time from implantation up to about 180 d during which it is highly unlikely that any materials leached from the polymer surface, and the period from 270 to 1280 d, where leachables cannot be discounted, especially towards the later time points. However, as shown in Table 1, the possible release of polymer degradation products during the later stages of the study did not have a noticeable effect on the frequency at which bone apposition or encapsulation responses were observed. The possible effect of leachable degradation products is therefore not a confounding factor in this study.

As expected for a degradable polymer, initial pin strength and stiffness decreased over time. When tested at 37°C, the ultimate tensile strength ranged initially from 16 ± 2.5 MPa for poly(DTO carbonate) pins to 36 ± 3 MPa for poly(DTE carbonate) pins. Poly(DTE carbonate) retained its strength for the longest period of time. Strength decreased in a linear fashion to about 10% of its initial value at 270 d post implantation. For poly(DTB carbonate), the majority of pins retained their strength up to about 180 d, while poly(DTH carbonate) and poly(DTO carbonate) became untestable within the first 90 d period. The initial Young's modulus of pins made of poly(DTE carbonate), poly(DTB carbonate), poly(DTH carbonate), and poly(DTO carbonate) was, respectively, 1000 ± 100, 700 ± 70, 500 ± 40, and 360 ± 40 MPa. In contrast to the variable degrees of strength retention, pin stiffness (as measured by Young's modulus) did not change significantly over the testing period.

Overall, poly(DTE carbonate) and poly(DTB carbonate) were very comparable in their physicomechanical properties (initial strength and stiffness, retention of strength and stiffness) but showed significantly different bone responses. Thus, physicomechanical properties can be excluded as key factors in the observed differences in the bone response.

### *3.4. Proposed mechanism for the observed differences at the bone–implant interface*

Factors such as the implant's biomechanical circumstances, chemical composition, mechanical properties, surface energy, surface roughness, and surface topography have all been implicated in affecting the bone response to an implant surface [21]. In this study we have controlled for many of these factors. Implants with identical shape and surface topography were placed in sites having comparable biomechanical environments. The observed differences at the bone–implant interface cannot be due to differences in bulk degradation of the polymers as the polymers degraded at comparable rates throughout the entire period of the study (Fig. 4). Further, during the first 180 d of the study none of the implants had yet degraded to the point of releasing detectable levels of any degradation products. Thus, the release of leachables can be excluded as a contributing factor for the different bone–implant responses. Similarly, variations at the bone–implant interface observed for poly(DTE carbonate) and poly(DTB carbonate) cannot be explained by the measured differences in pin mechanical strength or differences in stiffness during the degradation process. Thus, it seems that the changes in the chemical composition of the polymers must be a key factor responsible for the observed differences in the bone–implant interface.

Ertel and coworkers [11] analyzed the surface chemistry of the same series of tyrosine-derived polycarbonates and found that surface hydrophobicity increases, as measured by air–water contact angle, as the pendent chain is increased in length from ethyl (73°) to octyl (90°). In polymer films incubated in phosphate buffered saline,

Ertel also documented with attenuated total reflectance (ATR) FTIR that over a 40 week incubation period, ester pendent chains were cleaved via hydrolysis on the surface of each polymer. However, the surface ester pendent chains were hydrolyzed *significantly faster* for the more hydrophilic poly(DTE carbonate) surfaces than for the more hydrophobic poly(DTB carbonate) or poly(DTO carbonate) surfaces.

Therefore, poly(DTE carbonate) is distinguished from the other tyrosine-derived polycarbonates by the rate at which carboxylate groups (–COOH) are formed on the polymer surface. This phenomenon may be key to explaining why poly(DTE carbonate) surfaces exhibit more direct bone apposition than the other polycarbonate surfaces. Any two appropriately positioned pendent ester chains, when hydrolyzed to carboxylate groups, can form an ionic binding site for calcium ion. Similar to the observations reported for Polyactive®, the degrading tyrosine-derived polycarbonates have a potential for complexing calcium ions which in turn may act as nucleation sites for the formation of an inorganic hydroxyapatite surface layer. The hydroxyapatite layer may first lead to direct bone apposition and ultimately to bone bonding. While all tyrosine-derived polycarbonates exhibited some bone apposition, the higher frequency of bone apposition observed for poly(DTE carbonate) may be due to the increased rate of surface pendent chain hydrolysis and the increased likelihood of surface calcium chelation within the timeframe required to elicit bone apposition rather than encapsulation.

In support of this proposed mechanism, model polymer systems of partially hydrolyzed poly(DTE carbonate) were prepared with 0, 25, or 50% carboxylate groups, modeling poly(DTE carbonate) after 0, 25, or 50% of the ethyl ester pendent chains had hydrolyzed. After incubation at 37°C in simulated body fluid (SBF) for up to 14 d, solvent cast polymer films were analyzed with ESCA/XPS (Fig. 5). Over the short period studied, poly(DTE carbonate) surfaces did not chelate calcium because the insignificant amount of surface ester hydrolysis did not provide a sufficient density of calcium chelating sites. In contrast, the model polymer systems which had exposed carboxylate groups did chelate calcium ions, in relation to the concentration of carboxylate groups at the surface. Poly(DTE carbonate) modeled to have 25% of all pendent chains hydrolyzed was able to chelate up to 0.21% surface $Ca^{2+}$ by 7 d, and poly(DTE carbonate) modeled to have 50% of the pendent chains hydrolyzed, had chelated 0.67% surface $Ca^{2+}$ by 3 d.

Since the bone–polymer response is fully established within the first 180 d (Table 1), a polymer must hydrolyze rapidly enough to allow for the generation of a sufficient surface concentration of carboxylate groups to chelate calcium ions. The polymers with longer pendent chains, such as poly(DTB carbonate) and poly(DTO carbonate), have a slower rate of hydrolysis and consequently

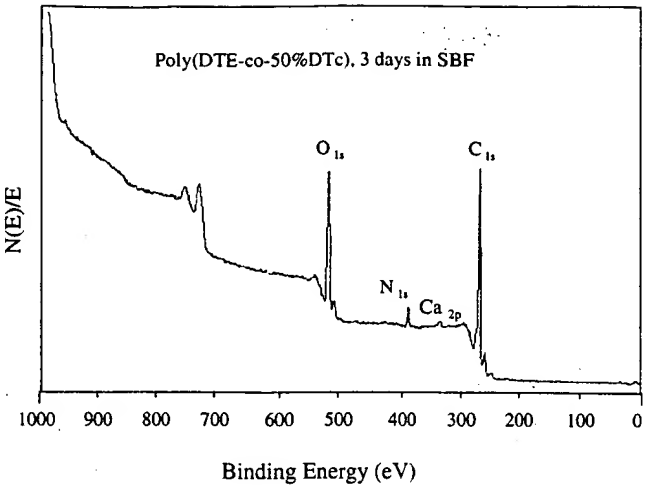


Fig. 5. ESCA/XPS analysis of a model polymer film, poly($DTE_{0.50}$-*co*-$DT_{0.50}$ carbonate). In this figure, the model represents a stage at which 50% of all ester pendent chains of the original poly(DTE carbonate) had been hydrolyzed. The results were obtained after the sample had been incubated in SBF at 37°C for 3 d. Note the $Ca^{++}$ peak between 300 and 400 eV.

a fibrous capsule was formed before enough carboxylate groups had formed. While all the tyrosine derived polymers meet the traditional definition of biocompatibility, poly(DTE carbonate) elicited direct bone apposition to the implant surface with the highest frequency and would obviously be a preferred material in the design of orthopedic implants.

## 4. Summary

The series of tyrosine-derived polycarbonates were found to be osteocompatible. These polymers, in particular poly(DTE carbonate), have physicomechanical properties that are comparable to other degradable polymers [22,23]. In comparing the bone–implant interface, pins of poly(DTE carbonate) exhibited direct bone apposition with a frequency greater than any of the other polycarbonates studied. Our results indicate that the observed differences in the frequency of direct bone apposition are a function of the rate at which the polymer pendent chain is hydrolyzed on the polymer surface. We postulate that the cleavage of a sufficient number of pendent chains imparts the ability to bind calcium ions to the implant surface which, in turn, leads to direct bone apposition and ultimately to bone bonding. This study highlights the importance that systematic variations in polymer chemistry can play in eliciting fundamentally different biological (bone) responses and it represents the first instance in which very small changes in the chemical structure of an implant material could be correlated with significant changes in the tissue response in a long-term, clinically relevant model. Our results are in line with an increasing

body of literature [8,24] that indicates that polymers can be designed to exhibit a bone bonding hard-tissue response if chemical binding/nucleation sites for calcium are presented at the polymer surface. The findings of this study illustrate the need for a careful optimization of the chemical structure of implant materials—an important point that is often overlooked by tissue engineers who have limited their studies to the use of the widely available polylactides or polyglycolides. Currently, the family of tyrosine-derived polycarbonates, in particular poly(DTE carbonate) is being explored in the design of bone fixation devices and suture anchors and as scaffolds for bone tissue engineering [19].

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